Modelling attention-deficit hyperactivity disorder: From hair follicles to neurons using induced pluripotent stem cells

Re, Silvano ; Werling, Anna ; Berger, Gregor ; Walitza, Susanne ; Grünblatt, Edna

Abstract: Despite tremendous research efforts, the exact causative mechanisms of attention-deficit hyperactivity disorder (ADHD) still have to be elucidated. One major challenge is the difficulty to reflect the complexity and simultaneously the individual features of ADHD with the current in vitro or in vivo model systems. A new strategy to circumvent this problem is to develop a patient specific research model of a «small personalized brain» in a dish using the induced pluripotent stem cell technology. This approach has now been initiated for the first time in Switzerland at the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of Psychiatry Zurich.

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Modelling attention-deficit hyperactivity disorder

From hair follicles to neurons using induced pluripotent stem cells

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Autor:
Silvano Re
Department of Child and Adolescent Psychiatry and Psychotherapy,
University Hospital of Psychiatry Zurich,
University of Zurich, Switzerland

Autor:
Gregor Berger
Department of Child and Adolescent Psychiatry and Psychotherapy,
University Hospital of Psychiatry Zurich,
University of Zurich, Switzerland

Autor:
Anna Maria Werling
Department of Child and Adolescent Psychiatry and Psychotherapy,
University Hospital of Psychiatry Zurich,
University of Zurich, Switzerland

Autor:
Prof. Dr. Edna Grünblatt
Department of Child and Adolescent Psychiatry and Psychotherapy,
University Hospital of Psychiatry Zurich,
University of Zurich, Switzerland
Neuroscience Center Zurich, University of Zurich and ETH Zurich, Switzerland
Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland
E-Mail: edna.gruenblatt@kjpd.uzh.ch

Autor:
Susanne Walitza
Department of Child and Adolescent Psychiatry and Psychotherapy,
University Hospital of Psychiatry Zurich,
University of Zurich, Switzerland
Neuroscience Center Zurich, University of Zurich and ETH Zurich, Switzerland
Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland

PSYCHIATRIE

Despite tremendous research efforts, the exact causative mechanisms of attention-deficit hyperactivity disorder (ADHD) still have to be elucidated. One major challenge is the difficulty to reflect the complexity and simultaneously the individual features of ADHD with the current in vitro or in vivo model systems. A new strategy to circumvent this problem is to develop a patient specific research model of a «small personalized brain» in a dish using the induced pluripotent stem cell technology. This approach has now been initiated for the first time in Switzerland at the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of Psychiatry Zurich.

Keypoints

The iPSC technology allows the generation of individual-specific brain cells and thus can be used to develop an in vitro model system for disorders affecting the brain.
Pluripotent stem cells can be generated from adult somatic cells harvested from patients and healthy controls without invasive procedures.
Subsequently, the cells can be forced to adopt neuronal or glial lineages and disease specific phenotypes can be identified using a multitude of different biochemical techniques.

ADHD is one of the most common psychiatric neurodevelopmental disorders in children with an estimated prevalence of over 5% in children and adolescents worldwide.[1] In more than half of affected individuals, at least some of the behavioural and cognitive deficits and the associated functional impairment persist into adulthood.[2] Since the core symptoms of ADHD adversely affect various aspects of life, ADHD can have drastic consequences on school and academic performances, interpersonal relationships and social integration. Thus, ADHD, when untreated, represents a substantial socioeconomic burden for the patient and his family and for the society.[3] Even though certain symptoms can be alleviated, a full remission of the symptoms is rarely reported.[4] Numerous lines of evidence suggest that the genetic component plays a substantial role in the etiopathogenesis of ADHD, showing a heritability higher than 70%.[5] Nevertheless, ADHD is not monogenetic but rather found to have a polygenic character, depicting the disorder as a heterogeneous complex phenotype constituted of multiple low penetrance susceptibilities.[6] In addition, environmental and socioeconomic factors have also been implicated in ADHD, as for example prenatal substance or lead exposures.[7] In other words, the etiology of ADHD is currently largely unknown despite extensive research efforts. Elucidating the underlying etiopathology of the disorder may lead to the discovery of biomarkers allowing a more precise and objective diagnosis as well as to identify biologically relevant subtypes. Such a biologically driven characterization of ADHD may finally improve the management of ADHD and potentially open the door to new therapy options.

Research approaches for ADHD

Genetic studies are fundamental resources to identify heritable susceptibilities of ADHD. However, investigating the contribution of those risk genes to the development of the disorder in humans is highly challenging, given that no direct manipulation can be performed. Furthermore, ADHD post-mortem brain samples are only rarely available and difficult to obtain for research purposes. So far, no study has been published in which ADHD post-mortem brain

tissue has been investigated. On the other hand, more accessible peripheral biomaterials, such as saliva or blood samples and skin cells, can be collected and studied at different time points. However, they lack the regional identity of neuronal cells. Neuroimaging techniques along with neuropsychological assessment are precious tools for the recognition of differences in brain anatomy, activity and functionality between patients and healthy controls. Nevertheless, they lack the resolution to individuate components behind the cellular cause of the disease at the molecular and biochemical level. Although some individual components of ADHD can be investigated with the help of a broad spectrum of models and technologies (see Fig. 1), no truly exhaustive way to study all aspects of the disorder has been established yet. In vivo model systems are often not sufficient to give useful insights into the molecular mechanism underlying neurodevelopmental and psychiatric disorders, mainly due to the more complex nature of the human nervous system and, in particular, the traits affected by the disorder which might be unique to humans. Thus, animal models, though very valuable, may not be suitable to fully embody complex and heterogeneous mental disorders, such as ADHD, and often exhibit only part of the whole spectrum of the disorder, which may as well arise from different etiologies. Notable efforts have been invested in the establishment of animal models using different approaches, ranging from genetic knockouts to chemically or environmentally induced models, in different animal species.[8] For example, the dopamine transporter (DAT) knockout mice[9] or inbred spontaneously hypertensive rats[10] show distinct behavioural features typical for ADHD such as hyperactivity. However, all these models do not fully mimic ADHD and the complex human brain. Lastly, in vitro applications using immortalized cell lines may also have questionable validity as a model for ADHD because they lack the genetic background of the individual patients and disregard the complexity of the whole organism. Although the aforementioned research approaches are very valuable for the understanding of neurodevelopmental disorders, there is still a necessity for a patient specific cellular model to investigate the molecular etiopathology of the disorder. In the last decade, increasing applications for the induced pluripotent stem cell (iPSC) technology have emerged, including the possibility to develop in vitro personalized neuronal systems derived from patients suffering from various disorders.

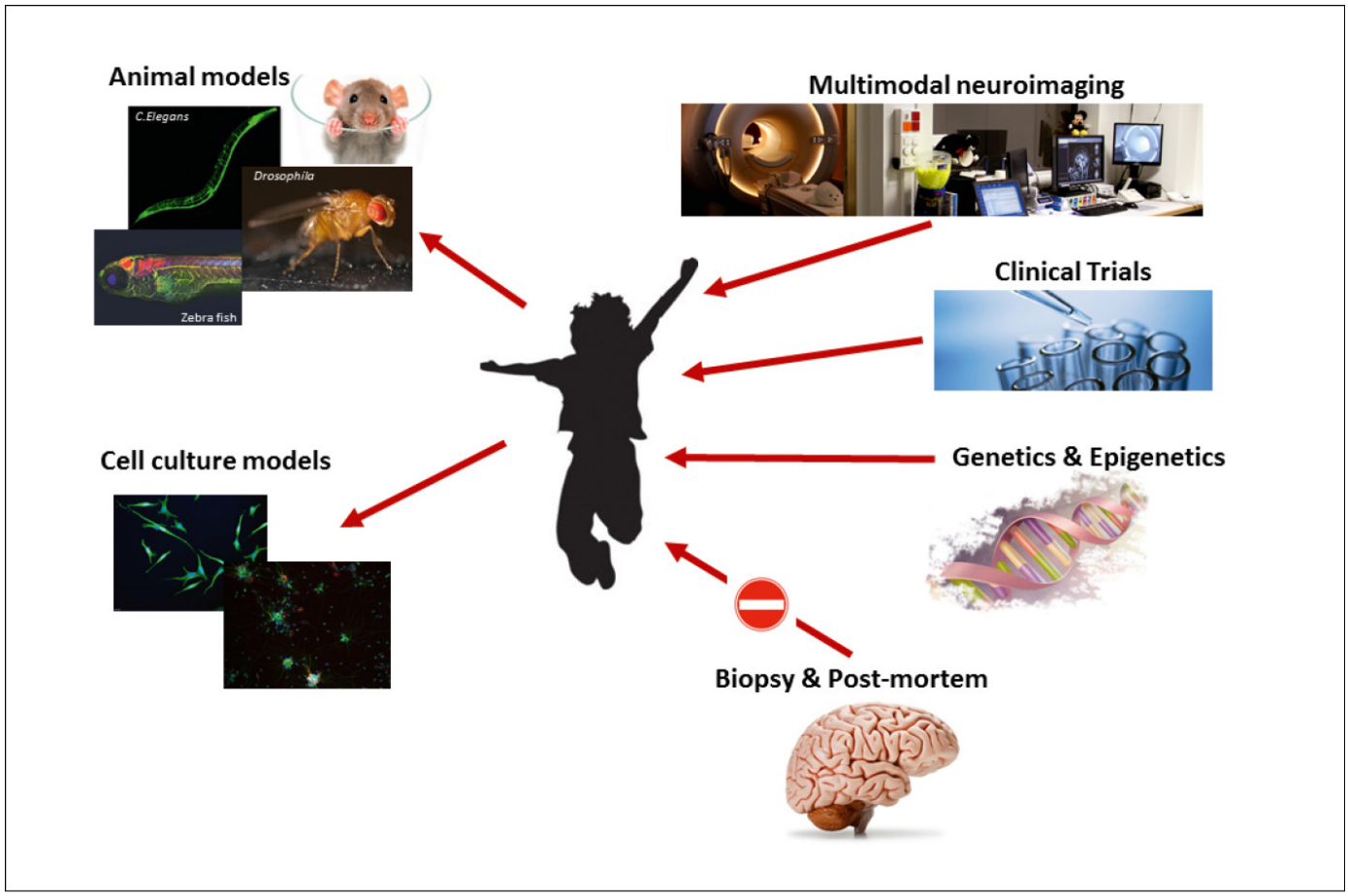

Fig. 1: Current approaches for the study of ADHD. A multitude of different approaches have brought useful insight about the disorder. However, a new model, which allows personalized characterization and direct modelling of the diseased tissue is needed

The iPSC technology

In 2006 Yamanaka and his colleagues succeeded to generate cells with embryonic stem cell features (i.e. self-renewal capability and pluripotency) from mouse fibroblasts.[11] Subsequently, they showed that the method can also be applied to human fibroblasts.[12] The generated cells were proved to be pluripotent, showing the potential to differentiate into every cell type and were accordingly termed „induced pluripotent stem cells" (iPSCs). Since then, many advances took place in the field, allowing the reprogramming of a multitude of different starting cell types into iPSCs with assorted approaches.[13] The technique of iPSC represents one of the greatest discoveries of the last decades in the field of cellular biology. This technology is not only extremely valuable for basic research and disease modelling, but also paved the way for new approaches in the field of drug screening and cell replacement therapy.[14] iPSC lines have been already established from a multitude of neurological and psychiatric disorders including late onset disorder such as Alzheimer's disease and Parkinson's disease, as well as early onset disorder such as Down syndrome, Rett syndrome and autism spectrum disorder.[15]

Modelling ADHD with iPSC

For the study of neurodevelopmental disorders, the reprogramming of cells extracted from a plucked hair follicle represents an ideal choice, since hair samples can be harvested with minimal distress to the affected child and thus avoiding invasive procedures such as skin biopsy or blood withdrawal. A multitude of various differentiation protocols has been already developed, allowing the generation of different subtypes of neuronal cells with distinct

regional brain identity. Most commonly, iPSCs are first differentiated into neural stem cells (NSCs), which still maintain the ability to self-divide and thus can be further expanded. Later, the cells can be terminally differentiated into post-mitotic neurons by providing the appropriate nutrition and signalling in the cell culture. [16] After a thorough characterization of the identity of the generated cell lines, they can be frozen and stored, as well as deposited in dedicated cell banks (e.g. European Bank for induced pluripotent Stem Cells; https://www.ebisc.org/), which allow the sharing of lines between various research groups. Other cell lineages, such as glial stem cells, may also be of interest for the study of ADHD.

Depending on the application, also more complex system can be employed. The generation of three-dimensional (3D) cellular structure in a dish offers a more faithful recapitulation of brain development since it is composed of several cell types with different regional identity and a more realistic spatial organization. However, such organoids are generally difficult to obtain and require a prolonged period of culture[17], while 2D models provide easy access to cellular mechanisms and more reproducibility.

As it is postulated that the molecular dysfunctions underlying ADHD occur during early development of the nervous system, it is particularly interesting to compare the dynamics of the differentiation and maturation into neuronal cells from patient specific stem cells and control lines. A multitude of experiments, such as proteomics, transcriptomics, metabolomic etc. analyses[18] and electrophysiology, can be performed to assess molecular abnormalities in ADHD specific lines. Ultimately, therapy approaches to address such abnormalities can be developed, which may open the door to the discovery of new therapeutic options (see Fig. 2).

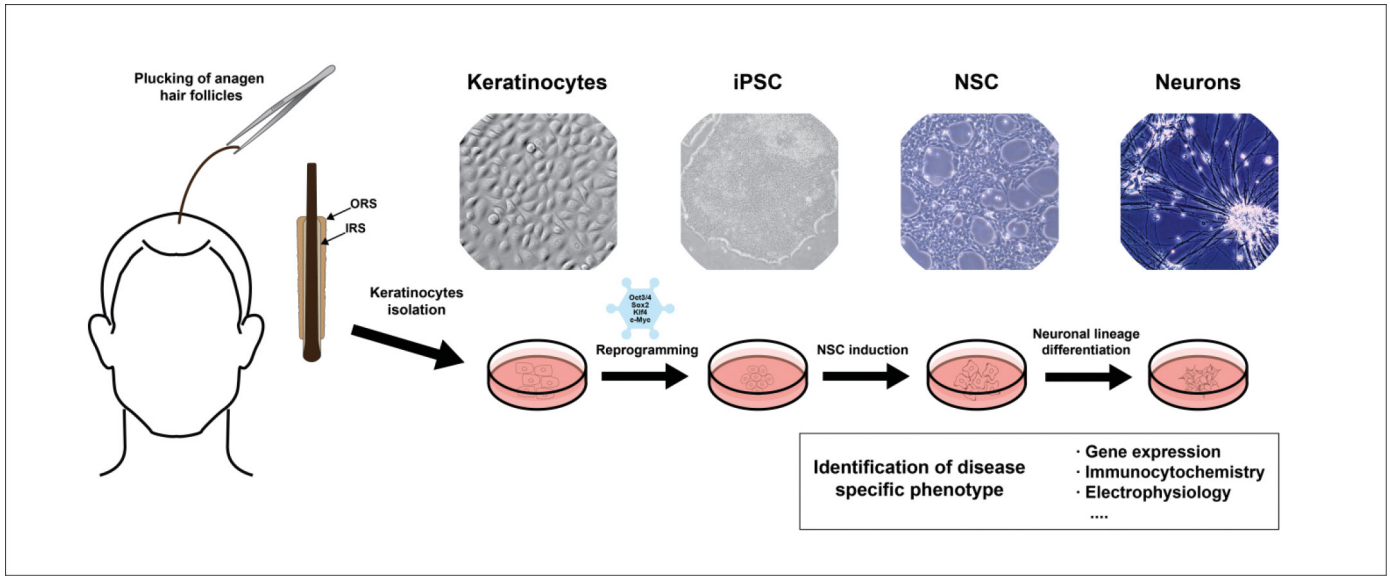

Fig. 2: Schematic outline of the workflow for the generation of personalized neuronal cell lines. Anagen hair follicles (hair follicles at the active growth phase) plucked from human scalp are processed to isolate keratinocytes, which are subsequently reprogrammed to iPSCs through the delivery of the Yamanaka's factors. The generated iPSC cultures are induced to adopt a neural lineage with fore- and midbrain identity by providing specific culture conditions

Conclusions

The research on ADHD benefits from a range of approaches that aim to uncover the cause of the disorder and to find new therapeutic options. Nevertheless, there is still a huge lack of knowledge of the molecular mechanisms underlying this neurodevelopmental disorder. Since it is not possible to study cells from the human brain directly, an alternative approach has to be identified. iPSCs can be generated from easily accessible cells, such as hair follicle-derived keratinocytes, and have the potential to differentiate in every cell type of the body. The differentiation of iPSC into neuronal cells recapitulates key points of neurodevelopment under controlled conditions, exposing a specific time frame of early central nervous system development. This technology is particularly suitable for disease modelling since the generated cells maintain the original genetic background of the donor and thus might expose disease specific deficits at the molecular level. Using gene editing technologies, it is as well possible to modify genetic components of interest and study the effects in vitro. The establishment of the here described personalized system, using in vitro neuronal culture or complex brain organoid, could contribute to fill the gap of knowledge behind ADHD research and eventually be used for the discovery of new pharmaceutical drugs.

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